

## ACTION OF ELECTROLYTES ON THE DORSAL MEDIAN NERVE CORD OF THE LIMULUS HEART

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The neurogenic origin of the heart beat of *Limulus* has been demonstrated by Carlson (1909) and confirmed by the more recent work of Garrey (1930) and Samojloff (1930). This demonstration depends upon the fact that the dorsal median nerve cord, where the rhythm originates, can be separated almost entirely from the heart musculature without interrupting their connection, so that experiments can be performed on the nerve and on the musculature separately. Although the literature on the effect of electrolytes on the vertebrate heart and on certain types of invertebrate heart has been very extensive since Ringer's time, relatively few papers have appeared (chiefly by Carlson himself) on the actions of salts on the *Limulus* heart. It seemed, therefore, highly desirable that further work of this kind should be carried out, especially in reference to the action of salts on the automatic nervous rhythm.

The present work was done on the nerve only. The dorsal median nerve cord (or ganglion) was isolated from the posterior end of the heart, remaining attached anteriorly, and the heart was sectioned after the second segment. This preparation, first used by Carlson (1906), is similar to a nerve-muscle preparation. The regular contraction of the heart depends upon the rhythmic nervous discharge, originating in the ganglion; and the amplitude of contraction depends upon the intensity of the nerve impulse; presumably this depends upon the number of nerve cells acting together. The first two segments of the heart were mounted in Ringer's solution and attached to a light heart lever for graphic registration with the nerve hanging down into the test solution. Any such solution could be quickly changed for another without disturbing the preparation.

Instead of using sea water as an indifferent medium for the ganglion, a simplified medium similar in composition to Ringer's solution and isotonic with the Woods Hole sea water ( $\Delta = 1^{\circ}.82$ ) was used. This modified Ringer's solution consisted of 100 vols. 0.52 M NaCl, 2 vols. 0.52 M KCl, and 15 vols. 0.29 M CaCl<sub>2</sub>, and proved highly satis-

factory as a substitute for normal sea water or *Limulus* plasma. In this medium both the nerve and the whole heart could be kept in good condition for several hours, although in some cases there was a slight temporary increase in rate during the first few minutes after transfer from sea water to the medium. The hydrogen-ion concentration of this solution was slightly on the acid side of neutrality (pH ca. 6); the same was true of the other salt solutions used in this work. Any effect due to the change of hydrogen-ion concentration was inappreciable in these experiments, except in a few cases to be described later.

Isotonic solutions were used throughout. When the salt concentration, considered by itself, was lower than isotonic, the solution as a whole was made isotonic by the addition of sucrose. According to Garrey's determinations (1915), 0.52 M NaCl, 0.29 M MgCl<sub>2</sub>, and 0.73 M sucrose are isotonic with the Woods Hole sea water, and hence with the Ringer's solution and the blood. All experiments were done at room temperature; this varied from 21° to 27° C. The action of a given salt solution was indicated by the change of activity observed in the heart muscle when the Ringer's solution surrounding the ganglion was exchanged for the experimental solution at the same temperature. All solutions were in gaseous equilibrium with the air and uniform in pH.

## THE ACTION OF SINGLE SALTS

### *Neutral Sodium Salts*

As shown by Carlson (1906a), the first effect of pure isotonic NaCl solution is to increase the rate of the nervous rhythm; this effect is followed by decrease in the intensity of the nervous discharge, as shown by lessened height of contraction, irregularity of rhythm, and ultimate standstill. The time for the abolition of the rhythm varies from half an hour to an hour or more. The height of contraction may increase somewhat at the beginning or may not. The invariable effects are (1) the rapid rate, (2) the irregularity of the rhythm and amplitude and (3) the gradual decrease and final cessation of contractions. All the other neutral sodium salt solutions have similar actions, differing only in degree. The quantitative differences between the neutral sodium salts follow the anion series of Hofmeister (lyotropic series); this can be demonstrated in several ways, as follows:

(a) By the relative effectiveness in increasing the rate of the nervous rhythm. When a nerve is transferred from Ringer's solution to a solution of pure NaCNS, for instance, the rate (contractions per minute) is only slightly increased, but the height of contraction falls off rapidly and the rhythm ceases in a minute or two. NaI has exactly the same action, except that the rate of increase is greater, and the rhythm

is maintained longer, usually for two to three minutes. A specific toxic or inhibitory effect of the pure Na-salts is thus shown, for when all electrolytes are removed by surrounding the ganglion with isotonic sucrose solution, the rhythm persists for some time, frequently for half an hour or more. The relative effectiveness of the different salts in increasing the rate shows the order of the Hofmeister series, NaCNS being the least and Na<sub>2</sub>SO<sub>4</sub> the most effective. Table I and Fig. 1 show the typical effect on the rhythm in the different solutions during the first five minutes after transfer from Ringer's solution, expressed as percentages of the original rates in Ringer's solution.

The data in Table I were obtained with a single heart. The ganglion was first treated with NaCNS solution until the rhythm ceased com-

TABLE I

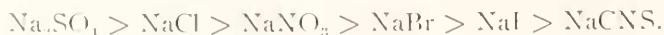
*Relative effectiveness of the neutral sodium salts in increasing the rate of rhythm. Numbers represent percentage increases in rate over the control, i.e.:*

$$\left( \frac{\text{rate in test solution} - \text{rate in Ringer}}{\text{rate in Ringer}} \times 100 \right)$$

	1st min.	2d min.	3d min.	4th min.	5th min.
NaCNS.....	18	stopped			
NaI.....	72	77	stopped		
NaBr.....	54	84	124	147	155
NaNO <sub>3</sub> .....	82	145	165	170	160
NaCl.....	96	129	170	205	235
Na <sub>2</sub> SO <sub>4</sub> .....	210	305	480		

pletely; the NaCNS was then replaced by Ringer's solution; rhythm, at first slow, was resumed, becoming normal in rate in a few minutes. The Ringer's solution was then replaced by the second solution in the series (NaI), and the beats were counted as before until cessation. Rhythm was again restored by return to Ringer's solution as before, and the next salt solution was used similarly, and so on in the order indicated in Table I. The series was then repeated with the same solutions in the reverse order; Ringer's solution being first replaced by Na<sub>2</sub>SO<sub>4</sub>, then by NaCl and so on. The effect of treatment for five minutes with the Na-salt solution was always perfectly reversible; in every case normal rhythm returned after transfer to Ringer's solution. The ganglion was apparently uninjured at the end of the series. Each figure in Table I thus represents the average of two determinations on the same ganglion.

The relative effectiveness of the different salts in accelerating the rhythm shows the order:



It is remarkable that, although NaCNS is the least effective in accelerating the rhythm, it is the most effective in causing standstill; *i.e.*, the order of relative toxic action of these salts is the reverse of the order of effectiveness in increasing the rhythm (with the apparent exception of  $\text{Na}_2\text{SO}_4$ , which differs from the others in being a calcium-precipitating salt). The same order of relative toxicity is also found with ciliary movement, another form of automatic rhythmic activity (Lillie, 1906, 1909; Höber, 1909), with spermatozoa and eggs (Gellhorn, 1927 *a* and

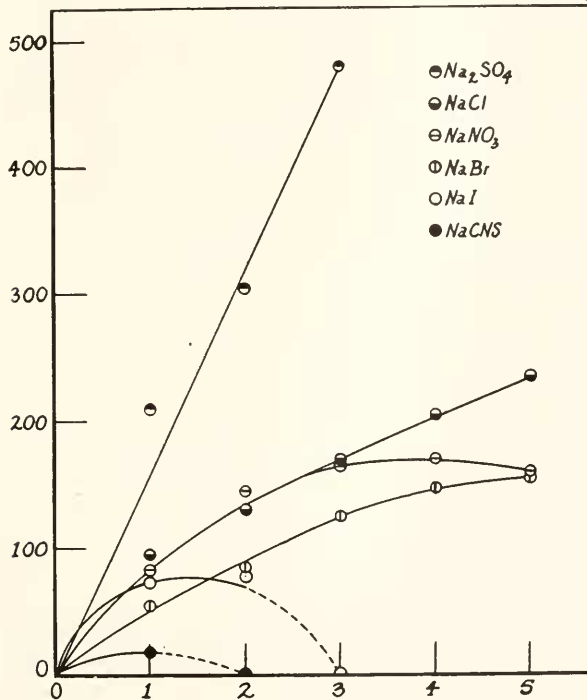


FIG. 1. Relative effectiveness of the neutral sodium salts in increasing the rate. Ordinate: percentage of increase in rate. Abscissa: time in minutes.

*b*), and with many other cells and tissues (Höber, 1926). In general, therefore, the order of toxicity of the series of Na-salts on *Limulus* nerve agrees very well with that observed in other tissues, as well as with the order of relative action on colloidal systems.

(*b*) The same order of relative toxicity is also shown when the NaCl in Ringer's solution is replaced by equivalent amounts of the other Na-salts. In such a modified Ringer's solution the ganglion shows a decline in activity, which is well marked at the end of ten minutes or sooner



and varies with the nature of the substituting salt. In Table II the effects observed in a typical series are summarized.

All the Na-salts, except  $\text{Na}_2\text{SO}_4$ , on replacing NaCl in Ringer's solution, decrease both rate and amplitude, and the order of relative effectiveness again follows the Hofmeister series.  $\text{Na}_2\text{SO}_4$  alone increases the rate, apparently because of its calcium-precipitating property. Calcium sulphate was actually seen to be precipitated in the solution. This decrease in calcium would naturally remove part of its antagonistic effect and increase the rate (see below).

In the frog's heart the replacement of NaCl in Ringer's solution by other Na-salts gives a similar series, but the order of relative action is reversed. While Na-salts of CNS, I, Br, and  $\text{NO}_3$  increase the amplitude, frequency, and pulse volume,  $\text{Na}_2\text{SO}_4$  decreases them (Handovsky,

TABLE II

*Changes in rate and amplitude on replacing NaCl in Ringer's solution by other Na-salts. Numbers give the percentage change of rate for 10 minutes as compared with normal rate in Ringer's solution.*

	Percentage change of rate	Amplitude
$\text{Na}_2\text{SO}_4$ .....	+30	gradually decreasing, about 2/3 normal at end of 10 minutes
NaBr.....	-18	slight decrease but regular
$\text{NaNO}_3$ .....	-34	more decrease and less regular
NaI.....	-37	rapid decrease and irregular
NaCNS.....	-46	decrease more rapid and more irregular

1923) (Sakai, 1914). On the theory that the quantitative differences in the physiological effects of the Na-salts are to be referred to their differences of action on the colloidal structures of the living tissue (Höber, 1926), it would appear that the colloidal structures directly affected by the salts differ in the *Limulus* nerve and in the vertebrate heart in such a way that the orders of relative action are opposed. Differences of this kind are not uncommon in living tissues (see below).

(c) The same series is also shown in *Limulus* nerve when we compare the relative effectiveness of the salts in initiating new rhythm after immersing the ganglion for varying lengths of time in solutions of  $\text{CaCl}_2$ , KCl, non-electrolyte, or in other unbalanced media which abolish the rhythm. To produce this effect isotonic  $\text{CaCl}_2$  was found to be the most convenient solution, since this salt is less toxic than KCl and is

more rapid in its effect than a non-electrolyte, such as sugar. When a nerve is transferred from Ringer's solution to pure isotonic  $\text{CaCl}_2$ , the rhythm ceases in about one minute. If the nerve be then transferred to a Na-salt solution, new rhythm is initiated sooner or later, the interval depending upon the time of immersion in  $\text{CaCl}_2$  and the nature of the Na-salt. The results of a typical series of such experiments are seen in Table III, in which are given the intervals required for the reappearance of the rhythm in the isotonic solutions of the various Na-salts after inhibiting the ganglion by immersion in isotonic  $\text{CaCl}_2$  for one to three minutes.

The relative effectiveness in initiating new rhythm follows the order

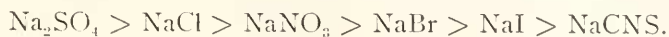


TABLE III

Intervals after which new rhythm appears in isotonic solutions of neutral Na-salts after inhibition by immersion in isotonic  $\text{CaCl}_2$  for varying lengths of time.

	Duration of $\text{CaCl}_2$ treatment		
	1 minute	2 minutes	3 minutes
sea water...			immediate recovery
$\text{Na}_2\text{SO}_4$ ....			less than 50 sec.
$\text{NaCl}$ .....		less than 30 sec.	after 55 sec.
$\text{NaNO}_3$ ....		after 35 sec.	after 2 min. and 15 sec.
$\text{NaBr}$ .....		end of 4th min.	after 3 min.
$\text{NaI}$ .....	35-60 sec.		no recovery in 5 min.
$\text{NaCNS}$ ....	end of 5th min.		no recovery in 5 min.

A balanced solution like sea water is more effective than any pure Na-salt solution.<sup>1</sup>

(d) It was shown by Schwarz (1907) and later by Gellhorn (1932, a and b) that if a frog's skeletal muscle be fatigued to loss of irritability by repeated stimulation while immersed in a solution of a given Na-salt (diluted with isotonic sucrose or Ringer's solution), it will recover irritability when transferred to pure solutions of certain other salts but not in all, the possibility of recovery depending upon the position of the salt in the Hofmeister series. If, for example, a muscle be fatigued in

<sup>1</sup> For restoring the rhythm in the frog's heart after standstill in isotonic sugar solution, Gellhorn (1932a) found  $\text{NaCNS}$  to be the most effective salt, the other Na-salts showing the lyotropic order of relative effectiveness but in the reverse order to that found in *Limulus* nerve.

$\text{NaNO}_3$ , it will recover in a solution of any salt on the right of  $\text{NaNO}_3$  in the series



but not in solutions of the salts on the left.

Similarly, when the rhythmic activity of a *Limulus* ganglion has ceased in a given pure Na-salt solution, new rhythm may, or may not, be restored on transfer to another Na-salt solution, according to the effectiveness of the salt in initiating new rhythm as found above. When the rhythmic nervous discharge has stopped in NaI solution, for example, new rhythm is initiated by NaBr or any other salt on the left of NaI in the above series, but not by NaCNS. If the rhythm is stopped in NaCNS solution, new rhythm is initiated by NaI and all the other salts. When the rhythm is stopped by  $\text{NaNO}_3$ , however, only NaCl and  $\text{Na}_2\text{SO}_4$  can initiate the new rhythm. This kind of experiment is best performed by abolishing the rhythm with NaCNS and NaI and then observing the recovery in the other salt solutions. For the other salts the rhythm is abolished only after a long time, and by this time the nerve is usually in bad condition. In such cases it is difficult to repeat the process of inhibition and recovery on the same nerve; for this reason the whole series has not been systematically investigated. The experiments so far performed have, however, shown uniformly that when a nerve has lost its rhythmic activity in a given salt solution, no recovery occurs in any other solution that is less effective than itself in initiating new rhythm. Here again the order of relative activity is the reverse of that found in the vertebrate tissue; some difference in the electrical properties of the structural colloids in the two cases is indicated.

In general we conclude that the effect of all pure Na-salt solutions on the activity of the ganglion is twofold: (1) a stimulating effect shown in the initiation of new rhythm and acceleration of the normal rhythm, and (2) a toxic effect shown in the decrease of amplitude of contraction and eventual standstill. The initiation and maintenance of rhythm are an important function of the Na-salts; no other salts, except Li-salts (see below), nor non-electrolytes can initiate new rhythm. Similarly, NaCl is a stimulating agent for the vertebrate heart in general (Lingle, 1900), for many invertebrate hearts and for the frog's lymph heart (Moore, 1901). It also can produce rhythmical stimulation in vertebrate muscle (Loeb, 1899) and nerve (Mathews, 1904), and it is equally important in the maintenance of the irritability of these tissues (Overton, 1902, 1904).

*Other Sodium Salts: Sodium Acetate*

This salt is not included in the series of neutral sodium salts since its solution has an alkaline reaction. In pure isotonic solution it has a pronounced stimulating effect on the nerve, increasing the rate several times in a few minutes; its efficiency in this respect is intermediate between that of  $\text{Na}_2\text{SO}_4$  and that of  $\text{NaCl}$ . It differs from the other Na-salts in that it rapidly decreases the intensity of nervous discharge, the amplitude of contraction being only about one-seventh of the normal at the end of the third minute; recovery also is slow and incomplete, indicating the infliction of some permanent injury upon the nerve.

*Calcium-precipitating Sodium Salts*

In general, all the calcium-precipitating salts (sulphate, fluoride, tartrate, and citrate) show the same type of influence on the *Limulus*



FIG. 2. Between the arrows, sea water was replaced by isotonic  $\text{Na}_2\text{SO}_4$  for three minutes. All figures read from left to right.

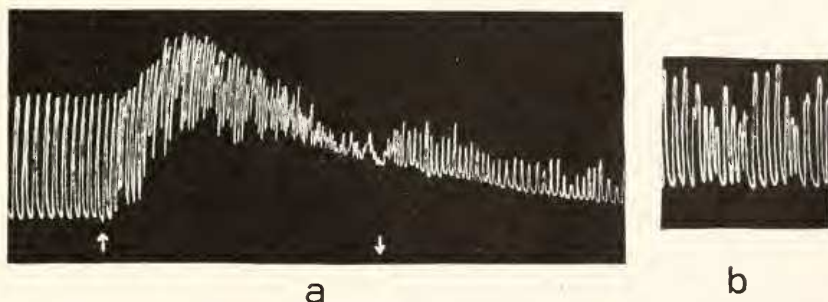


FIG. 3. (a) Between the arrows, Ringer's solution was replaced by isotonic sodium tartrate for two minutes.

(b) After 43 minutes in Ringer's solution.

nerve; all produce at first a rapid rhythmical discharge, shown by irregular contractions and tetanus in the muscle, followed by cessation and muscular relaxation within one or two minutes. The stimulating effectiveness, as shown by the rapidity of the initial rhythm and the completeness of the tetanus, is greatest with citrate and least with

sulphate; tartrate and fluoride are intermediate. The depressant or toxic effect, as measured by the time required for recovery in Ringer's solution, is greatest with tartrate; then come (in the order) fluoride, citrate, and sulphate (see Figs. 2, 3, 4, and 5).<sup>2</sup>

*Lithium Chloride*

In general LiCl has an action similar to that of NaCl, but is decidedly more toxic. The initial increase in rhythm is much greater and is followed in a few seconds by irregularity. On returning the ganglion to Ringer's solution recovery is very slow, requiring half an hour

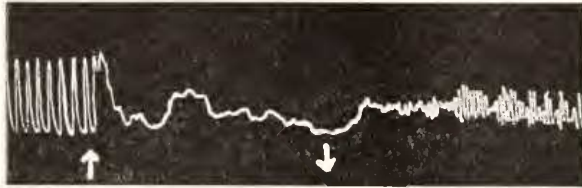


FIG. 4. Between the arrows, Ringer's solution was replaced by isotonic sodium citrate for one minute.

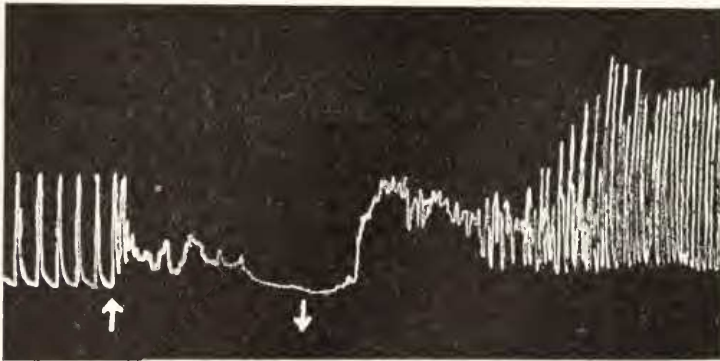


FIG. 5. Between the arrows, Ringer's solution was replaced by isotonic NaF for one minute.

or more to restore the normal rhythm. LiCl can also initiate new rhythm but is far less effective than NaCl; typically, after treating the ganglion with  $\text{CaCl}_2$  for three minutes, NaCl can initiate new rhythm in one minute, while LiCl requires at least three minutes. When a nerve that has failed to recover after three minutes in LiCl is transferred into NaCl, new rhythm starts immediately. Like the other neutral sodium salts, LiCl can not replace NaCl in Ringer's solution without harmful effect.

<sup>2</sup> The intense stimulating action of this group of calcium-precipitating salts is also seen in frog's nerve (Mathews, 1904) and in skeletal muscles of various kinds (Garrey, 1905).

*Potassium Chloride*

Carlson (1906*d*) found that when the ganglion is immersed in pure isotonic KCl there is an immediate increase in the height of the first one or two contractions; this is followed by a feeble but rapid rhythm and standstill in a minute or less. A primary stimulating effect and an inhibitory effect of KCl are thus distinguishable. The stimulating effect is particularly evident when KCl is present in high concentration in Ringer's solution. Inhibition by KCl is perfectly reversible if the nerve is not subjected to its action too long. Recovery in normal Ringer's solution, however, is always slower than after Ca-inhibition, and a rapid rhythm always appears at the beginning of the recovery.

*Salts of Alkali Earth Metals*

Of the commonly used salts of the alkali earth metal group, chlorides of Ca, Mg, and Sr have similar action with certain differences of degree. Pure isotonic BaCl<sub>2</sub>, however, as first shown on skeletal muscle by Loeb (1899), on vertebrate nerve by Mathews (1904) and on the muscle and nerve of the *Limulus* heart by Carlson (1906*d*), has an intense primary stimulating effect, somewhat like that of KCl, producing a rapid initial

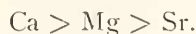
TABLE IV

Average change in rate for 5 minutes (expressed as percentages of the original rate in Ringer's solution) following the addition of varying amounts of CaCl<sub>2</sub>, MgCl<sub>2</sub>, and SrCl<sub>2</sub> to Ca-free Ringer's solution.

	Vols. of isotonic alkali earth salt solutions added to 100 vols. isotonic NaCl and 2 vols. KCl				
	0	5	15	30	50
MgCl <sub>2</sub> .....	+82	+41	+32	+ 3	-14
CaCl <sub>2</sub> .....		+22	0	-11	-25
SrCl <sub>2</sub> .....		+17	-12	-21	-31

rhythm followed by rapid inhibition. When the CaCl<sub>2</sub> in Ringer's solution is replaced by an equal amount of BaCl<sub>2</sub>, the rhythm is increased in rate and becomes irregular.<sup>3</sup>

Chlorides of Ca, Mg, and Sr in pure isotonic solutions decrease both the rate and the intensity of the nervous discharge, without any indication of a primary stimulation, and arrest the rhythm in a few minutes. The order of relative effectiveness in arresting the rhythm is



<sup>3</sup> AlCl<sub>3</sub> has a similar action, causing rapid initial rhythm and rapid inhibition. Solutions of AlCl<sub>3</sub> have, however, an acid reaction.



Typically, in experiments on the same nerve, Ca arrests the rhythm in about one minute, Mg in about two minutes, and Sr within three minutes. When the salts are added in varying quantities to Ringer's solution surrounding the ganglion a retardation of rhythm is also seen, but the order of relative effectiveness is different. Table IV gives the percentage change in rate of one nerve during five minutes when the ganglion is placed in Ca-free Ringer's solution (*i.e.*, a mixture of 100 vols. 0.52 M NaCl plus 2 vols. 0.52 M KCl) to which varying proportions of the

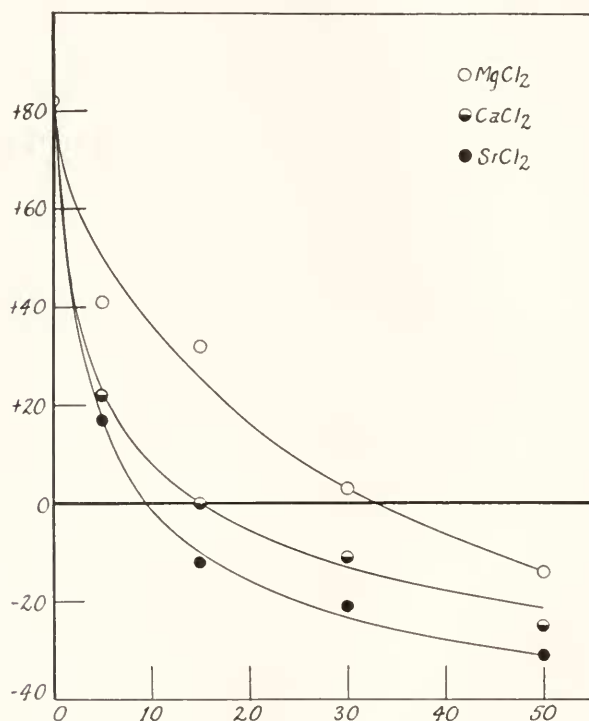


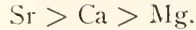
FIG. 6. Comparison of varying amounts of  $MgCl_2$ ,  $CaCl_2$ , and  $SrCl_2$  in Ringer's solution. Ordinate: average percentage of change in rate for five minutes. Abscissa: volumes of isotonic solutions of the alkali earth salts added to 100 vols. NaCl and 2 vols. KCl.

three salts are added, as compared with the control rate in normal Ringer's solution.

The calculations on the average percentage change in rate were made as follows. When the nerve was immersed in a solution of 100 vols. isotonic NaCl plus 2 vols. isotonic KCl, the rhythm increased from 17 beats per minute in Ringer's solution to 23, 31, 32, 33, 36 in the successive minutes. The average increase in rate for the five minutes was

from 17 to 31, or an increase of 82 per cent. All the calculations for percentage change in rate were made in the same way.

It is evident from Table IV and Fig. 6 that the relative effectiveness of the three cations in decreasing the rate under these conditions follows the order



Kisch (1927*b*) found the same series in a study of the influence of these salts on the frequency of the vertebrate heart.

It might be expected that for each of the three salts a certain concentration could be found at which the normal rhythm would be maintained as in Ringer's solution. According to the curves of Fig. 6, about thirty volumes of  $\text{MgCl}_2$ , or ten volumes of  $\text{SrCl}_2$ , should have the same effect as the 15 volumes of  $\text{CaCl}_2$ . This is, however, not exactly the case. For example, in the mixture of 30 vols.  $\text{MgCl}_2$ , 100 vols.  $\text{NaCl}$ , and 2 vols.  $\text{KCl}$  there was actually a slight increase in rate for the first two minutes, followed by a continuous gradual decrease to a value below normal. Evidently  $\text{MgCl}_2$  by itself is unable to make a balanced solution with  $\text{NaCl}$  and  $\text{KCl}$  in spite of the fact that magnesium is normally more concentrated in sea water than calcium. If  $\text{MgCl}_2$  is used together with  $\text{CaCl}_2$  in the normal proportion of sea water ( $\text{Mg}/\text{Ca}$  ca. 3), the normal rhythm is maintained quite well. This inability of  $\text{MgCl}_2$  to replace  $\text{CaCl}_2$  in Ringer's solution was also observed by Kisch (1929) and by Goljachowski (1932), using the vertebrate heart. In the *Limulus* ganglion  $\text{SrCl}_2$  was more favorable than  $\text{MgCl}_2$  as a substitute for  $\text{CaCl}_2$  in Ringer's solution; yet even under the best conditions a gradual decrease in rate was also found. Apparently neither  $\text{MgCl}_2$  nor  $\text{SrCl}_2$  can completely replace  $\text{CaCl}_2$  in Ringer's solution.

#### SPECIFIC RÔLES OF $\text{NaCl}$ , $\text{CaCl}_2$ , AND $\text{KCl}$ IN RINGER'S SOLUTION AND THEIR ANTAGONISM

As we have seen,  $\text{NaCl}$  appears necessary for the maintenance of the ganglionic rhythm; but in pure isotonic solution of this salt the rhythm is rapid and irregular and cannot be long maintained. When to this solution  $\text{CaCl}_2$  alone is added to a concentration equal to that present in normal Ringer's solution, the rhythm becomes regular and is greatly reduced in rate, although still remaining above normal, while the amplitude of the contraction is increased somewhat. But even so the normal rhythm cannot be maintained, and the amplitude gradually decreases. It is only after the addition of the normal content of  $\text{KCl}$  in Ringer's solution that the medium becomes capable of maintaining the normal rhythm for a long time (see Fig. 7). A nerve can be kept in good condition for more than six hours in this balanced solution with

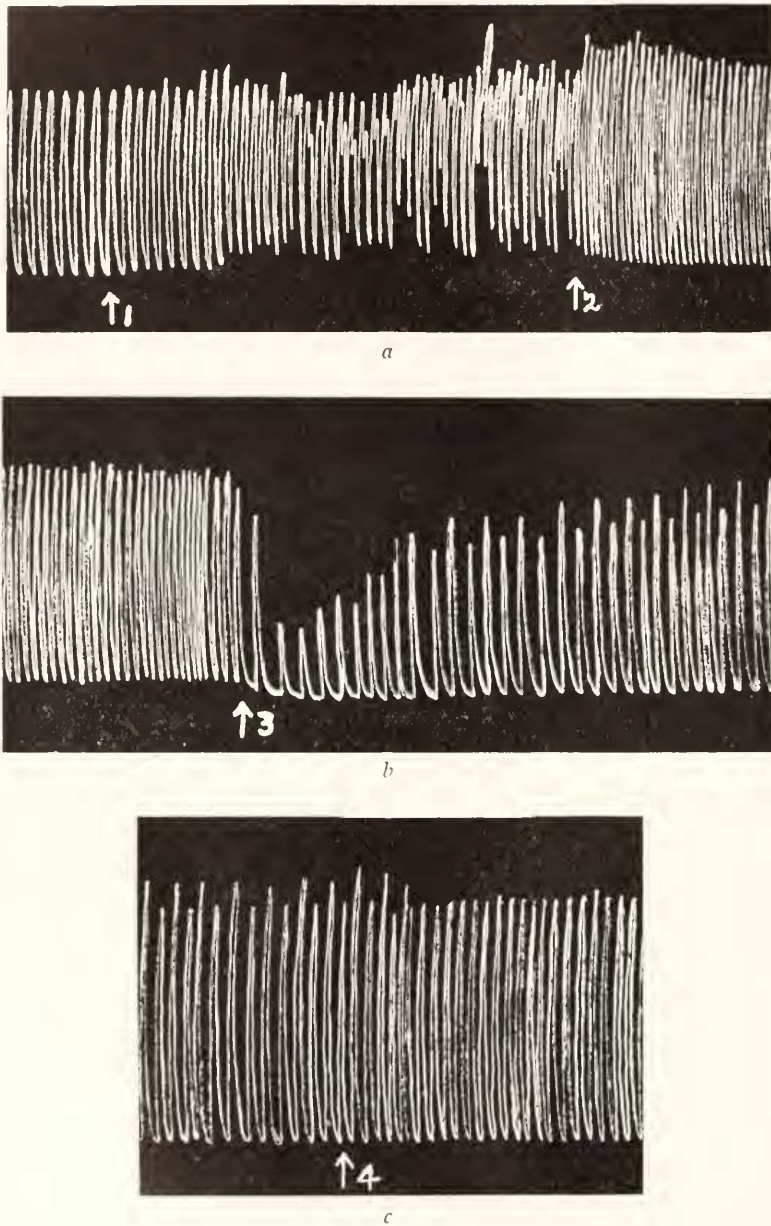


FIG. 7. Effect of transferring a ganglion from Ringer's solution.

(a) To pure isotonic NaCl at 1 for two minutes, then to a mixture of 100 vols. NaCl plus 30 vols. CaCl<sub>2</sub> at 2.

(b) Three minutes later, to a mixture of 100 vols. NaCl plus 30 vols. CaCl<sub>2</sub> and 4 vols. KCl at 3, showing the potassium-paradox.

(c) Nine minutes later, back to Ringer's solution again at 4.

only a very gradual decrease in the rate and amplitude of the heart beat. It seems, therefore, that although  $\text{CaCl}_2$  antagonizes part of the toxic effect of pure  $\text{NaCl}$  the antagonism is by no means complete. Apparently the remaining part of the total effect of the  $\text{NaCl}$  is antagonized by  $\text{KCl}$ . Whether or not  $\text{KCl}$  antagonizes  $\text{CaCl}_2$  in normal Ringer's solution then becomes a question. It will, however, be shown below that there is no definite antagonism between  $\text{K}$  and  $\text{Ca}$  at their normal concentration in Ringer's solution. Working with marine organisms, Rubinstein (1926, 1927) has come to a similar conclusion; namely, that  $\text{NaCl}$  has two different toxic actions, one antagonized by  $\text{K}$  and the other antagonized by  $\text{Ca}$ , and that in the ordinary balanced solution there is no antagonism between  $\text{K}$  and  $\text{Ca}$ .

A striking phenomenon is observed when a nerve is transferred from the solution containing only  $\text{NaCl}$  and  $\text{CaCl}_2$  to the normal Ringer's solution containing also  $\text{KCl}$ . Instead of returning to its normal rhythm at once, either the rate or the amplitude (or both) is still further decreased, or in some cases the rhythm may cease altogether, and then after an interval return gradually to normal. This peculiar phenomenon has been described in the case of the frog's heart by Libbrecht (1920, 1921) and is called by him the potassium-paradox. It is obtained when the potassium-free perfusion fluid is exchanged for normal Ringer's solution, and is believed to be purely a potassium effect. Working on the rabbit, Busquet (1922, *a* and *b*) came to the opinion that the potassium-paradox is not a direct potassium effect but is connected with vagus stimulation. This idea has been refuted by Kisch (1927*a*). The production of potassium-paradox on the *Limulus* nerve also shows its independence of vagus action. The work on the vertebrate heart has shown that the effect depends on several factors: (*a*) on the difference in the potassium content of the two solutions (Libbrecht, 1921) (Witanowski, 1926), (*b*) on the duration of the perfusion with the  $\text{K}$ -free or  $\text{K}$ -poor solution (Kisch, 1927*a*), and (*c*) on the presence of a certain proportion of  $\text{Ca}$  (Kisch, 1927*a*). In general, the conditions are similar in the *Limulus* ganglion. The same type of potassium-paradox effect is obtained not only on changing from a  $\text{K}$ -free solution to Ringer's solution but also on changing from Ringer's solution to a solution of higher  $\text{K}$ -content. It is more readily obtained after a prolonged exposure to the  $\text{K}$ -free solution, or after repeated immersion in it. It is not obtained on changing from a pure isotonic sucrose solution to a sucrose solution containing  $\text{KCl}$  in its normal concentration in Ringer's solution, indicating that the presence of some other electrolytes is necessary. The exact conditions under which the  $\text{K}$ -paradox can be produced in the *Limulus* nerve have not been worked out in detail. It is interesting to note that Kisch (1930) has also found a similar phe-

TABLE V

Average change in rate for 5 minutes (in percentage of control rate) on addition of NaCl to 2 vols. KCl plus 15 vols. CaCl<sub>2</sub>.

Vols. NaCl added	200	150	100	50	10	0
Per cent rate change . . . . .	+96	+30	0	-8	-27	-36 *

\* Average rate for the last two minutes of rhythm (beat becomes inappreciable in five minutes).

nomenon with Ca and Sr (Ca- and Sr-paradox). The general nature of the paradox suggests that the deficiency of a certain ion (*e.g.* K-ion) produces a change in the tissue of such a kind that the subsequent addition of a small amount of the ion gives rise to its specific effect in an exaggerated form.

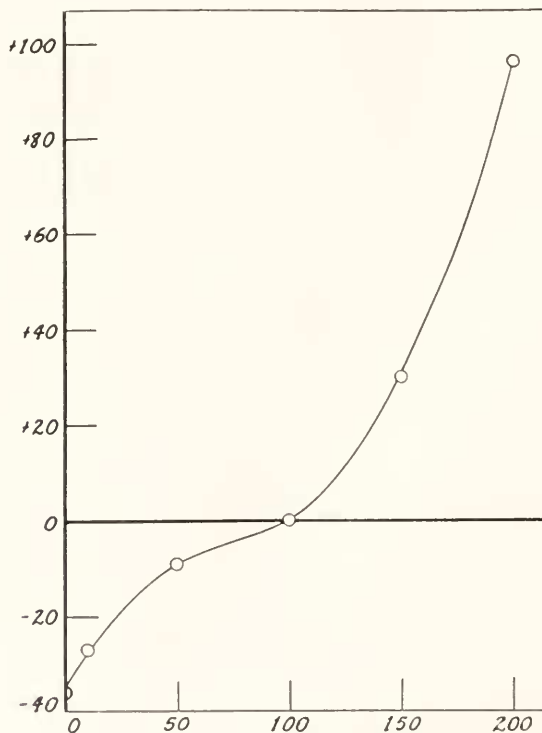


FIG. 8. Effect of varying the amount of NaCl in Ringer's solution. Ordinate: average percentage of change in rate for 15 minutes. Abscissa: vols. of isotonic NaCl added to 15 vols. CaCl<sub>2</sub> and 2 vols. KCl.

The specific rôles of the three salts in Ringer's solution can be studied by varying the concentration of each of them separately.

*Effect of Varying the NaCl Content of Ringer's Solution*

The normal Ringer's solution for *Limulus* heart contains 100 vols. 0.52 M NaCl, 15 vols. 0.29 M CaCl<sub>2</sub>, and 2 vols. 0.52 M KCl. If, keeping constant this ratio of CaCl<sub>2</sub> and KCl, one increases the relative concentration of NaCl, a rapid rate and some irregularity appear, characteristic of the action of unbalanced NaCl. When NaCl is present in a smaller ratio than 100 vols., the rhythm is retarded, showing the effect of excess of Ca and K. Table V and Fig. 8 show the effect of varying the relative concentration of NaCl in Ringer's solution.

*Effect of Varying the CaCl<sub>2</sub> Content of Ringer's Solution*

In general, increase in the proportion of Ca in Ringer's solution decreases the rate of beat. Table VI gives the average results of three experiments.

The curves in Fig. 9 show the antagonism between NaCl and CaCl<sub>2</sub> beyond any doubt. As regards NaCl and CaCl<sub>2</sub>, it is found that when the ratio of Ca:Na is less than 0.28 the rate is increased, indicating the predominance in the action of NaCl. When the ratio is greater than 0.28 the rate is decreased, showing a predominance of CaCl<sub>2</sub>. No mixture containing only NaCl and CaCl<sub>2</sub> can maintain the normal rhythm

TABLE VI

Percentage of change in rate for 5 minutes on addition of CaCl<sub>2</sub> (1) to 100 vols. NaCl and (2) to 100 vols. NaCl plus 2 vols. KCl.

Vols. CaCl <sub>2</sub> added	(1) to 100 vols. NaCl	(2) to 100 vols. NaCl and 2 vols. KCl	(1) - (2)
0	+123	+92	31
15	+ 27	0	27
30	- 5	-25	20
50	- 30	-46	16

for long; a certain amount of KCl is indispensable. This is also shown by the lower curve in Fig. 9, giving the effect of the addition of CaCl<sub>2</sub> to a mixture of 100 vols. NaCl and 2 vols. KCl. When the proportion of CaCl<sub>2</sub> is less than 15 vols. the rate is increased; with more than 15 vols. it is decreased. The parallel course of the two curves is remarkable, showing a nearly constant influence of the KCl. There is no indication of any direct antagonism between Ca and K. Each of these cations appears to antagonize its own fraction of the toxic effect of pure NaCl, quite independently of the other.

*Effect of Varying the KCl Content of Ringer's Solution*

As mentioned above, in a solution containing 2 vols. 0.52 M KCl plus 100 vols. 0.52 M NaCl the rate is slower than in pure NaCl. If



more KCl is added, the rate begins to increase again (upper curve, Fig. 10). A curve of similar shape is obtained on the addition of KCl to a mixture of 100 vols. NaCl and 15 vols.  $\text{CaCl}_2$  (lower curve, Fig. 10). Changing the KCl content of Ringer's solution in either direction (above or below the normal concentration of K) always results in an increase of rate. When the KCl content is decreased below normal, the rapid rate is due to the excess of NaCl; when the KCl is increased above normal, the acceleration results from the direct stimulating action of the K-ions. Accordingly we found that the toxic effect of the pure NaCl solution can be partly antagonized by the addition of 2 vols. KCl to 100 vols. NaCl;

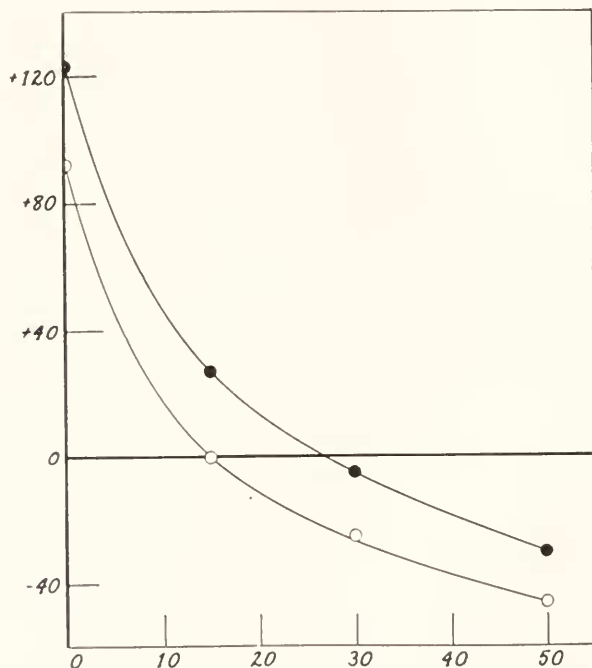


FIG. 9. Effect of  $\text{CaCl}_2$ . Ordinate: average percentage of change in rate for 5 minutes. Abscissa: vols. of  $\text{CaCl}_2$  added (1) to 100 vols. NaCl (upper curve) and (2) to 100 vols. NaCl plus 2 vols. KCl (lower curve).

while if more KCl is added its own stimulating effect is manifested. The effects are found in the presence of the normal amount of  $\text{CaCl}_2$  in Ringer's solution, indicating again the independent antagonistic actions of Ca and K in relation to NaCl (lower curve, Fig. 10). The differences of rate corresponding to the points on the two curves are seen in column four of Table VII, showing the nearly constant effect of addition of 15 vols.  $\text{CaCl}_2$  to each of the solutions in column three (i.e., the

addition of a definite quantity of KCl has the same influence on the rhythm, whether it is added to the pure NaCl or to the Ca-containing NaCl solution).

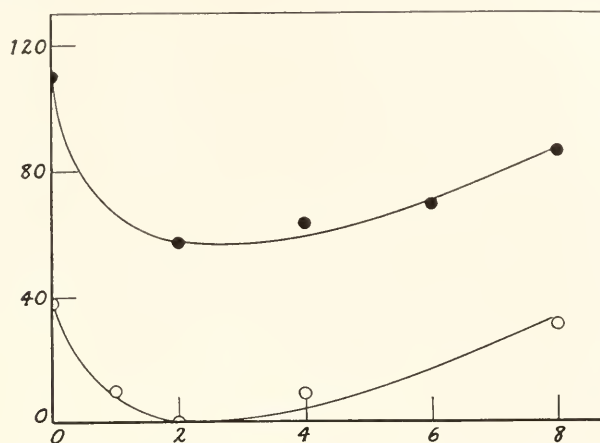


FIG. 10. Effect of KCl. Ordinate: average percentage of change in rate for 5 minutes. Abscissa: vols. KCl added (1) to 100 vols. NaCl (upper curve) and (2) to 100 vols. NaCl plus 15 vols.  $\text{CaCl}_2$  (lower curve).

#### *Effect of Varying KCl and $\text{CaCl}_2$ Together*

Since an excess of KCl in Ringer's solution increases the rate and an excess of  $\text{CaCl}_2$  decreases it, when both salts are present in excess we should expect to find typical antagonism. This may be tested ex-

TABLE VII

Percentage of change in rate for 5 minutes on addition of KCl (1) to 100 vols. NaCl and (2) to 100 vols. NaCl plus 15 vols.  $\text{CaCl}_2$ . Observations made on different nerves.

Vols. KCl added	(1) to 100 vols. NaCl	(2) to 100 vols. NaCl and 15 vols. $\text{CaCl}_2$	(1) - (2)
0	110	38	72
1	68 *	10	58
2	57	0	57
4	63	9	54
6	69	18 *	51
8	86	31	55

\* Found from the curves in Fig. 10.

perimentally by adding a certain definite excess of KCl to Ringer's solution; varying additions of  $\text{CaCl}_2$  are then made to this solution and the resulting changes in rate during the first five minutes are deter-

mined. When the action of K overbalances that of Ca the rate increases; when the Ca effect predominates the rate decreases. Table VIII shows the results of a series of such experiments obtained on three nerves.

TABLE VIII

Average percentage change in rate for 5 minutes on addition of  $\text{CaCl}_2$  to Ringer's solution containing varying additions of KCl.

Vols. KCl added to 100 vols. Ringer	Vols. $\text{CaCl}_2$ added to 100 vols. Ringer									
	0	10	20	30	40	50	60	70	80	
3	+22	- 2	-16							
6	+38	+15	+ 2	-8	-16					
9			+18	+5	- 5	-11				
12					+10	0	- 9			
18							+14	+4		-4

If the average changes in rate are plotted against the addition of  $\text{CaCl}_2$ , a characteristic group of curves is obtained (Fig. 11). For

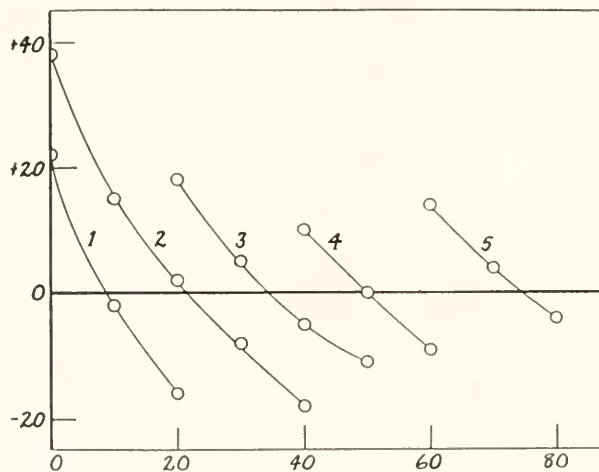


FIG. 11. Effect of addition of KCl and  $\text{CaCl}_2$  together. Ordinate: average percentage of change in rate for 5 minutes. Abscissa: vols.  $\text{CaCl}_2$  added to (1) 3 vols., (2) 6 vols., (3) 9 vols., (4) 12 vols. and (5) 18 vols. KCl plus 100 vols. Ringer's solution.

every concentration of KCl there should be a corresponding concentration of  $\text{CaCl}_2$  at which the two salts will more or less completely antagonize each other, *i.e.* at the intersections of the curves with the abscissa. For example, in a mixture of 3 vols. KCl solution with 100

vols. Ringer's solution, about 9 vols.  $\text{CaCl}_2$  should be added to antagonize the excess of KCl; with an addition of 6 vols. KCl, 21 vols.  $\text{CaCl}_2$  should be required, and so on as represented in Table IX.

The Ca/K ratios in this table are obtained directly from the curves in Fig. 11. In general the ratio seems to increase as the absolute amounts of Ca and K become higher. If, however, the Ca/K ratio is calculated on the basis of their total concentration in Ringer's solution (by adding approximately 2 vols. to the added excess of KCl and 15 vols. to that of  $\text{CaCl}_2$ ) the ratio becomes very nearly constant, the average being about 4.6. This indicates that Ca and K antagonize each other most completely at a definite ratio of 4.6, when they are present in excess in Ringer's solution. The minimal amounts of 15 vols.  $\text{CaCl}_2$  and 2 vols. KCl are those required to antagonize the toxic effect of 100 vols. NaCl. Apparently only the excess of K is directly antagonized by

TABLE IX

Vols.  $\text{CaCl}_2$  and KCl solutions necessary for complete antagonism of each other.

(1) added to 100 vols. Ringer			(2) added to 100 vols. NaCl		
CaCl <sub>2</sub>	KCl	CaCl <sub>2</sub> : KCl	CaCl <sub>2</sub>	KCl	CaCl <sub>2</sub> : KCl
9	3	3.0	24	5	4.8
21	6	3.5	36	8	4.5
34	9	3.8	49	11	4.5
50	12	4.2	65	14	4.6
75	18	4.2	90	20	4.5

the Ca (and vice versa). The value 4.6 should not be taken too strictly, for it does vary from nerve to nerve. Somewhere between 4 and 5 is a fair estimation on the whole. It should be added that the antagonism is not complete; a gradual decrease in the amplitude of contraction is always found. Evidently the three salts in Ringer's solution are so interrelated that it is only when they are present in the normal ratio that the solution forms a well balanced medium.

#### LOSS OF RHYTHM AND LOSS OF IRRITABILITY

##### *Minimal Concentration of Ringer's Solution for Maintenance and Initiation of Rhythm*

When a nerve is immersed in pure  $\text{CaCl}_2$  solution, the rhythmic nervous discharge stops in a minute or two, but the nerve can still be stimulated by a tetanizing current, as shown by the contraction of the heart. During the next several minutes (the exact time depending on

the condition of the nerve) the irritability gradually declines and finally disappears completely, so that the nerve is no longer stimulated by a current of any strength. The dissociation of automatic rhythm and irritability is particularly obvious in some cases. Occasionally, when a nerve is dissected out, it may not show any rhythmic activity at all, although it always responds to the tetanizing current and to a pure NaCl solution. When a nerve with its irritability abolished in isotonic CaCl<sub>2</sub> is transferred into Ringer's solution, the rhythm appears gradually, together with the associated irritability. It is difficult to determine by experiment whether the irritability or the rhythmic activity returns first, for usually an automatic rhythm is started by any brief stimulation during the recovery period. In all cases, however, where definite rhythm is restored, the nerve responds to electrical stimulation; *i.e.*, automatic rhythm is always associated with irritability.

It was found by Overton (1902, 1904) that a minimal amount of NaCl is necessary for the maintenance of irritability in vertebrate muscle and nerve (about 20 vols. isotonic NaCl plus 80 vols. isotonic sucrose). The minimal amount of Ringer's solution necessary for the maintenance of irritability in the *Limulus* nerve has not yet been determined. A mixture of 33 vols. Ringer's solution plus 67 vols. isotonic sucrose solution can maintain the normal rhythm for more than one hour with only a slight increase in rate at the beginning followed by a slow progressive decline in rate and intensity. The initial stimulating effect is due to the specific action of sucrose (Carlson, 1906c). The unfavorable influence of the lowered salt concentration is clearly shown when after an immersion of one hour in the dilute Ringer's solution the ganglion is returned to Ringer's solution of normal concentration. The rate and intensity are at once increased. A solution containing 10 vols. per cent Ringer's solution in isotonic sucrose solution can not maintain the normal rhythm; both the rate and the intensity of the rhythmic impulses decrease gradually, and the rhythm stops in about twenty minutes (*i.e.*, in about the same time as in pure sucrose solution). Similarly, when the rhythm of a nerve is inhibited by CaCl<sub>2</sub>, it recovers in a mixture of 30 vols. Ringer's solution plus 70 vols. isotonic sucrose. It appears, therefore, that this solution can maintain the normal rhythm of *Limulus* nerve for some time although not indefinitely, and can initiate new rhythm in a quiescent nerve.

#### DISCUSSION

The precise conditions determining the rhythmical activity of the nerve cells in the *Limulus* ganglion are not clearly understood at present, but the analogy of Lillie's iron wire model (Lillie, 1929) suggests

that some kind of automatic rhythmic breakdown and restoration of membrane structure in these cells is the primary physical change. The rhythmic activity is closely dependent on the environmental conditions, such as temperature (Carlson, 1906*a*), the osmotic pressure of the medium (Carlson, 1906*b*), and particularly the chemical composition of the medium. The nerve appears, however, to be comparatively indifferent to decrease of oxygen tension (Newman, 1906).

One very striking property of the ganglion is the promptness with which it reacts to the presence of electrolytes; in this respect it presents a contrast with the heart muscle. While isotonic NaCl solution stimulates the ganglion almost instantaneously, the heart from which the nerve cord has been removed develops a rhythmic series of contractions only after a prolonged immersion in the same solution, *e.g.* for 30 to 45 minutes (Carlson, 1905). In vertebrate muscle and nerve a contrast of the opposite kind is seen; the frog's sartorius shows rhythmic twitchings in isotonic NaCl solution immediately after immersion (Loeb, 1899), while under the same conditions the sciatic nerve gives rhythmic impulses only after two hours or more (Mathews, 1904). The extreme sensitiveness of the *Limulus* heart ganglion to the action of salt solutions is probably to be referred chiefly to the presence of nerve cells, although the non-myelinated structure of the fibers may also be a factor.

The action of ions on the ganglion is, on the whole, quite comparable with that observed on the vertebrate heart. Some differences in the electrical properties of the structural colloids of the tissue are, however, suggested by the reversed order of the lyotropic series, described in the first part of this paper. Another difference between the two tissues is seen in the well-established antagonism between K and Ca in the vertebrate heart (Ringer, 1882, *a* and *b*), as contrasted with the independent antagonisms of KCl and of CaCl<sub>2</sub> in reference to NaCl in the *Limulus* heart. The peculiar effect of CaCl<sub>2</sub> in arresting the vertebrate heart in systole, while with KCl the arrest is in diastole, may be attributed to special structural and chemical peculiarities of this heart. In the *Limulus* heart, every contraction is the index of a train of nerve impulses originating in the ganglion. If there is no impulse from the ganglion, the heart remains in a condition of complete relaxation. CaCl<sub>2</sub> abolishes the nerve impulse without any sign of stimulation, leaving the heart muscle in a relaxed condition.

In the foregoing description, less attention has been paid to changes in amplitude of contraction than to changes in rhythm for the reason that the former show less consistency than the latter, except in those cases cited above. The changes in rate are definite and reproducible and can be conveniently used as a criterion for the evaluation and comparison of

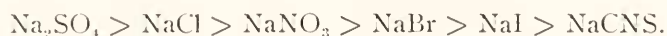


the actions of the electrolytes. In most cases the recorded changes of rate have reference to the first five, or sometimes the first ten to fifteen, minutes after transfer from one solution to another; but these rates can not be maintained unaltered in an imperfectly balanced medium. When a nerve is immersed in an unbalanced solution for a long time, some kind of harmful effect always results and recovery is incomplete.

#### SUMMARY

A Ringer's solution, containing 100 vols. 0.52 M NaCl, 15 vols. 0.29 M  $\text{CaCl}_2$ , and 2 vols. 0.52 M KCl, has been found satisfactory as a substitute for sea water or *Limulus* blood plasma in maintaining the normal rhythmic activity of the ganglion and heart.

Neutral sodium salts in isotonic solutions exhibit (1) a stimulating effect shown by the initiation of new rhythm in a quiescent nerve and an increase in the rate of the rhythm, and (2) a toxic effect shown by the production of irregularity and early cessation of the rhythm. The relative effectiveness of the salts in initiating new rhythm and increasing the rate follows the order



The toxicity follows the reverse order.

LiCl can also initiate new rhythm but is much less effective than NaCl and more toxic.

Other sodium salts, acetate, fluoride, tartrate, and citrate, have an intense stimulating action due either to their specific toxic action, or to their calcium-precipitating property, or to the alkaline reaction of some of them.

Chlorides of Ca, Mg, and Sr inhibit the rhythm with gradual decrease in rate and intensity. The relative effectiveness in decreasing the rate follows the order



$\text{BaCl}_2$  has a primary stimulating effect but inhibits the rhythm rapidly.

Pure isotonic KCl has both a primary stimulating effect and an inhibitory effect, producing a rapid rate followed by early cessation of the rhythm. At its normal concentration in Ringer's solution, it antagonizes about one-fourth of the toxic effect of the NaCl; at higher concentrations than normal it exhibits at first a stimulating or accelerating effect and later an inhibitory effect.

$\text{CaCl}_2$  at its normal concentration in Ringer's solution antagonizes about three-fourths of the toxic effect of the NaCl; at higher concen-

trations than normal it produces an inhibitory effect, decreasing the rate and amplitude.

Ca and K antagonize each other at a definite proportion (about 4 or 5 to 1) when both are present in excess in Ringer's solution.

Potassium-paradox has been observed on changing from a potassium-free solution to normal Ringer's solution.

The normal rhythm can be maintained by Ringer's solution of 30 per cent normal concentration (made isotonic by sucrose) and new rhythm can be initiated by the same solution. The rhythmic activity of the ganglion is more readily abolished than its responsiveness to electrical stimulation.

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